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A Novel Four Base-Pair Deletion Within the Aγ-GLOBin Gene Promoter Associated With Slight Increase of Aγ Expression in Adult

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We studied a Chinese family and revealed 5.4% and 3.2% fetal hemoglobin (HbF) with advantageously Aγ type in the mother and the daughter, respectively, using alkali denaturation assay and urea–Triton–acrylamide gel electrophoresis and high-performance liquid chromatography. The father's HbF was less than 0.5%. Large deletion was not observed within the β-globin gene cluster by restriction endonulease mapping. Characterization by the polymerase chain reaction (PCR) and DNA sequencing demonstrated the mother is a homozygote with a novel four base-pair "AAGC" (-226 to -223) deletion within the Aγ-globin gene promoter and the daughter is a heterozygote with this deletion. The deletion was not detected in the father. No any mutations were identified in the Gγ promoter of all the subjects studied. We propose that the small deletion is associated with the slight increase of Aγ gene expression in adult. Am. J. Hematol. 63:16–19, 2000. © 2000 Wiley-Liss, Inc.

Key words: human fetal globin gene; hereditary persistance of fetal hemoglobin; small deletion; promoter mutation

INTRODUCTION

In normal human development, fetal hemoglobin (HbF, $\alpha_2\gamma_2$) production switches to adult hemoglobin (HbA, $\alpha_2\beta_2$) production soon after birth. At the end of the first year of life, HbF constitutes less than 1% of the total Hb in red blood cells. The exact mechanisms involved in the switching have not been completely elucidated. Natural mutants that are associated with increase HbF production have provided models for the studies of the switching mechanisms.

HPFH (hereditary persistence of fetal hemoglobin) is a condition associated with higher levels of HbF synthesis (1.6–30%) during adult life without other hematological abnormalities in affected heterozygotes [1]. Molecular analysis has revealed that HPFH is caused by either large deletions 3′ to the γ-globin genes or point mutations (including small deletions) 5′ from the cap site of γ-globin gene promoters, classified deletional and non-deletional HPFH, respectively.

So far, many point mutations and a few small deletions have been identified in either Gγ or Aγ-globin gene promoter. These include -202 C→G, -200 +C, -175 T→C, -161 G→A, -158 C→T, -114 C→G, and -110 A→C in Gγ-globin gene promoter and -202 C→T, -198 T→C, -196 C→T, -195 C→G, -175 T→C, -117 G→A, -114 C→T, and -114 to -102 13 bp deletion in Aγ-globin gene promoter [2]. It is suggested that these mutations may be responsible for expression of the corresponding γ-globin gene in adult.

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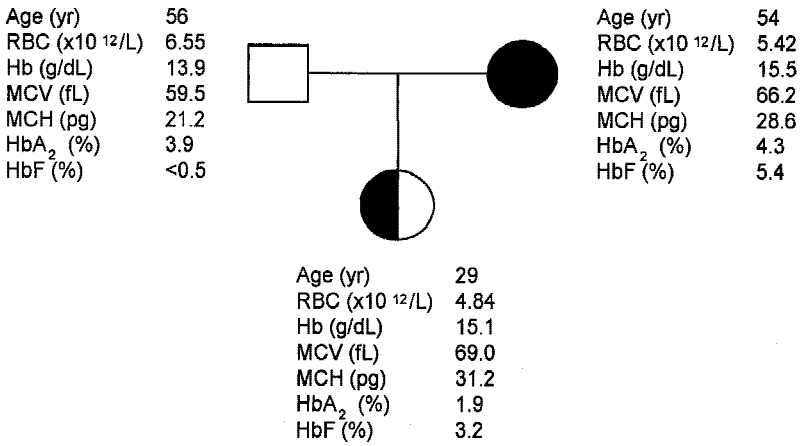

Fig. 1. Pedigree of the Chinese family with their hematological data.

We describe here a novel 4 bp deletion from -226 to -223 (AAGC) of the Aγ-globin gene that is associated with slightly elevated Aγ-globin production in two members of a Chinese family.

MATERIALS AND METHODS

Hematological Analyses

Blood samples from this family were collected with EDTA as the anticoagulant. Basic hematological indices were assayed by standard method. HbF was measured by alkali denaturation [3]. HbA_2 was quantitated by DE-52 microcolumn chromatography [4]. The relative proportion of Gγ, Aγ, β, and α chain were determined by urea–Triton–acrylamide gel electrophoresis [5] and by high-performance liquid chromatography (HPLC) using a large-pore Vgdac C4 column [6].

DNA Analyses

The genomic DNA was prepared from peripheral blood leukocytes and restriction endonuclease mapping was done as described [7]. Haplotypes including eight restriction fragment length polymorphism (RFLP) sites were analyzed. These RFLP sites include two *Hin*dIII sites in the Gγ and Aγ genes, *Xmn*I site 5′ to the Gγ gene, two *Hin*cII sites in the Ψβ region, *Hin*fI site 5′ to the β gene, and *Hpa*I 3′ to the β gene. The *Hin*dIII sites were determined by Southern hybridization using the Aγ 3.3 kb fragment as probe. Analyses of the other sites were performed by the PCR-based method [8].

Both the Aγ and Gγ promoter regions of the subjects were amplified from -562 (Aγ) or -563 (Gγ) to +244. The 5′ specific primer for Aγ promoter is 5′-GGCAAAAGTCACAAAGAGTA-3′ (-562 to -543) and for Gγ is 5′-GGCCTAAAACCACAGAGAGT-3′(-563 to -544). The common 3′ primer is 5′-TGACAGTCAGAAGGTGCCAC-3′(+225 to +244). One microgram of genomic DNA was mixed with 30 pmol of each primer, 200 μmol of dNTPs in a 100 μl reaction volume containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, and 5 units of Taq DNA polymerase. Amplification was carried out on a DNA Thermal Cycler 480 (Perkin Elmer). The DNA was denatured at 94°C for 5 min, and followed by 30 cycles of PCR. Each cycle of PCR consisted of denaturation at 94°C for 1 min, 30 s of annealing at 54°C, and 45 s of polymerization at 72°C. The final elongation step was carried out at 72°C for 10 min.

The amplified fragments were recovered from low-melting-temperature agarose gels and then blunt end ligated to the *Sma*I site of pGEM-4Z vector. The sequencing primer for both the recombinant plasmids and the purified fragments was 5′-CCAGAAGCGAGTGTGTG-GAA-3′ (+14 to -6 of γ-globin genes). The sequencing reactions were carried out using ABI PRISM™ Dye Primer Cycle Sequencing Ready Reaction Kit (Perkin-Elmer). Nucleotide sequence was assayed on ABI PRISM™ 377 DNA sequencer installed with Sequencing Analysis 2.1 software.

RESULTS

During a survey of hemoglobinopathies in a district of Southern China, a slightly elevated HbF was detected in two members of a Chinese family (Fig. 1). Electrophoresis on urea–Triton–acrylamide gel showed that the Gγ-globin chain in all the three members studied and the Aγ-globin in the father were almost undetectable but the Aγ/β+γ-globin chain is 5% and 3% in the mother and in the daughter, respectively. Similar results were obtained by HPLC measurement. The HPLC revealed that the Aγ/Aγ+Gγ is 89.5% in the mother and 83.4% in the daughter, respectively. A hypochromia (MCH 21.2 pg) and microcytosis (MCV 59.5 fl) and higher HbA_2 (3.9%) suggested that the father was a heterozygote of β-thalassemia. The slightly low MCV (66.2 fl) and higher HbA_2 (4.3%) suggested that the mother was probably a heterozygote of β-thalassemia. However, the normal MCH

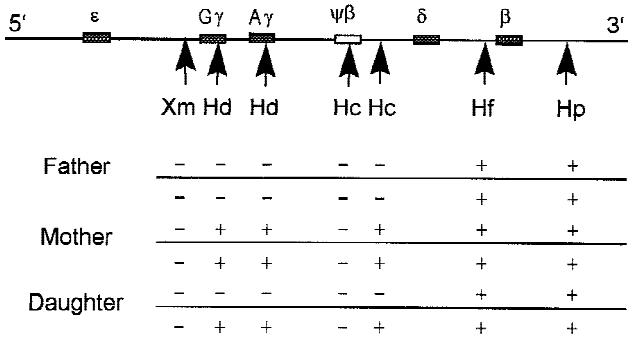

Fig. 2. The seven polymorphic restriction enzymes sites of the β-globin gene cluster analyzed in the present study. Xm, *Xmn*I; Hd, *Hin*dIII; Hc, *Hin*cII; Hf, *Hin*fI; Hp, *Hpa*I.

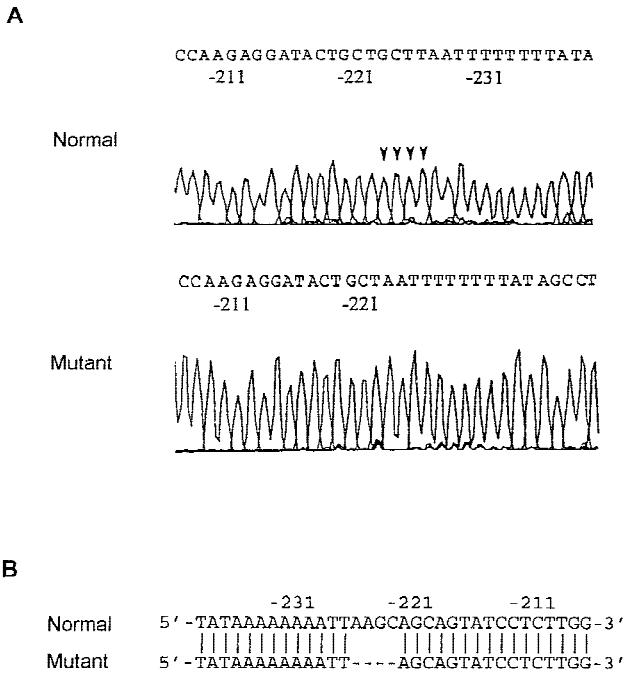

Fig. 3. DNA sequences surrounding the deletion region of the normal and mutant Aγ-globin gene promoter. (A) The DNA sequence of the antisense strand for the normal and mutant Aγ gene promoter from -207 to -239. The 4 bp deletion is 5′-GCTT-3′ from -223 to -226. The arrows indicate the deleted nucleotides. (B) DNA sequence of the sense strand for the normal Aγ gene promoter from -239 to -207 and the corresponding mutant promoter region. The 4 bp deletion is 5′-AAGC-3′ from -226 to -223.

(28.6 pg) was probably benefited from her higher HbF level. No any phenotypic appearance of β-thalassemia was identified in the daughter. The data of the daughter are consistent with the classic description of HPFH.

Restriction enzyme analysis of the β-globin gene cluster did not reveal any obvious DNA rearragement. The haplotypes including seven polymorphic sites are listed in Fig. 2. From the haplotype analysis, it can be confirmed that the haplotype "− + + − + + +" in the daughter is inherited from the mother and is related to the increased Aγ-globin gene expression.

Nucleotide sequence analysis of amplified Aγ-globin gene promoter showed that a novel 4 bp deletion occurred at position -226 to -223 in the mother and the daughter but not in the father (Fig. 3). This mutation was readily confirmed by cutting the amplified fragment with resriction endonuclease *Fnu*4H I, because the deletion abolished the recognition site from -224 to -219 of the normal Aγ-globin gene promoter. Combination of direct sequencing of the amplified fragment and sequencing of recombinant plasmids revealed that the mother is a homozygous and the daughter is a heterozygous for this mutation. The father's Aγ gene promoter is normal. No any mutation was identified in the Gγ gene promoter of all the subjects. The nucleotide at -158 of Gγ promoter is C instead of T in all the three subjects. It has been reported that the nucleotide T at -158 of the Gγ gene is probably associated with higher Gγ gene expression in some cases [9].

DISCUSSION

In this report we have described a novel deletion (AAGC, -226 to -223) in the Aγ-globin gene upstream promoter associated with small increased in vivo HbF. This deletion occurs in the region of two GCA repeats at -224 to -219 of the normal Aγ promoter. Short deletion are frequently associated with such short direct repeats and "slipped mispairing" during DNA replication is the likely mechanism for this kind of deletions [10]. Another 4 bp deletion (AGCA, -225 to -222) in the same region has been reported by Gilman et al. [11]. The subject in their study was a β-thalassemia with HbF 6.7% and Gγ 66.7%. The higher Gγ/Aγ ratio was hypothesized to be due to a decreased Aγ expression resulted from the 4 bp deletion, but it may also be explained by that an increasing anemic stress preferentially stimulates the Gγ-globin gene [12]. By analyzing quantitatively the globin chains in the β^0-thalassemia individuals who had higher HbF levels (HbF > 3%), Nanca and Gelman et al. further proved that the expression level of the Aγ-globin gene with the 4 bp deletion ($A\gamma^T$) was lower than that of the normal Aγ gene lacking the 4 bp deletion [13]. In the normal adults, HbF is usually lower than 1% of the total Hb and the Aγ/Gγ+Aγ is about 60%. In the β-thalassemia [11, 13] cases, the Aγ/Gγ+Aγ was 33% [11] and 38% [13] that were indeed lower than the normal, but the HbF with Aγ type was still slightly higher than the normal adult (<0.6%) because the β-thalassemia patients had higher HbF levels. The exact mechanisms responsible for the high level of the Gγ and Aγ product in some β-thalassemia cases are not clear yet, nor have any cases been provided to show how the -225 to -222 deletion

influences the the Aγ-globin gene expression in the non-β-thalssemia adult.

In our report here, the 4 bp deletion occurs in just a different position (-226 to -223) of the Aγ promoter. The increased HbF in the mother and daughter is almost Aγ type and the Aγ value in the mother who is a hemozygote with the deletion is about two times of that in the daughter who is a heterozygote with the deletion, suggesting that the -226 to -223 deletion is probably responsible for the increased Aγ expression.

Several DNA-binding proteins interacting with the γ promoter region have been identified and some point mutations that produce the HPFH phenotype occur in the binding motifs. Some of these mutations appear either to decrease or to increase transacting factors binding or to generate new binding site [14–21]. It is thought that the altered interactions between various transacting factors and the promoter may be responsible for the higher levels of HbF synthesis persisting into adult life.

At least three DNA-binding proteins, including Oct-1, GATA-1, and SATB1, have been shown to interact with the -200 to -240 region of the γ promoter [22–24]. In vitro studies have demonstrated the formation of bimolecular and trimolecular complex involving these proteins. The nucleotide at -223 is hypersensitive to DNase I in the nuclear extracts from K562 and Hela cells [22]. These suggest that this region may form a point of association of the β-globin gene cluster domain with the nuclear matrix and as such may act as a boundary or matrix-associating region in the regulating gene expression in the normal chromosome [1,25]. Our study provided a new proof that the mutation in this DNA region indeed influence the γ-globin gene expression.

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